Pure competition of multiple species during mixed-substrate microbial growth: Extending the resource-based theory.

Atul Narang

Department of Chemical Engineering, University of Florida, Gainesville, FL 32611-6005.
narang@che.ufl.edu

Sergei S. Pilyugin
Department of Mathematics, University of Florida, Gainesville, FL 32611-8105. pilyugin@math.ufl.edu

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Abstract

The simultaneous growth of multiple microbial species is a problem of fundamental ecological interest. In media containing more than one growth-limiting substrate, multiple species can coexist. The question then arises: Can single-species data predict the existence and stability of mixed-culture steady states in mixed-substrate environments? This question has been extensively studied with the help of resource-based models. These studies have shown that the single-species data required to predict mixed-culture behavior consists of the growth isoclines and consumption vectors, which in turn are determined from single-substrate data by making specific assumptions about the kinetics of mixed-substrate growth. Here, we show that these assumptions are not valid for microbial growth on mixtures of substitutable substrates. However, the theory can be developed by determining the growth isoclines and consumption vectors directly from the mixed-substrate data, thus obviating the need for specific assumptions about the kinetics of mixed-substrate growth. We show furthermore that in addition to the growth isoclines and consumption vectors, the single-species, mixed-substrate data yields a new family of curves, which we call the *consumption curves*. Consideration of the growth isoclines and the consumption curves yields deeper insights into the behavior of the mixed cultures. It yields *a priori* bounds on the substrate concentrations achieved during coexistence, permits the extension of the theory to systems in which the growth isoclines are non-monotonic, and clarifies earlier results obtained by considering only the growth isoclines.

1 Introduction

In natural water bodies and in many man-made bioreactors, multiple microbial species thrive in environments containing multiple growth-limiting substrates. These microbial species interact in diverse and complex ways, so that the development of a general theory encompassing all types of interactions is a daunting, if not hopeless, task. We can begin to make some progress by focusing on systems in which the interaction between the species is *indirect*, i.e., the specific growth and nutrient uptake rates of the individual species do not depend on the densities of the microbial species [7, 9]. However, the problem remains formidable even if we restrict ourselves to indirect interactions. For instance, the behavior can be quite complex, if one or more species excrete metabolic products that stimulate or inhibit the growth of the other species. If we ignore such indirect interactions stemming from excretion, we arrive at the problem of *pure* or *resource competition*. This work is concerned with the theory of pure competition between multiple microbial species.

We shall assume that the species in question are growing in a well-stirred chemostat supplied with a sterile feed having a fixed flow rate and composition. Natural and man-made water bodies are spatially heterogeneous, and constantly perturbed by fluctuations in the composition, concentrations, and flow rates of the nutrients. Yet, we make this idealization because among all the processes occurring in ecosystems, the

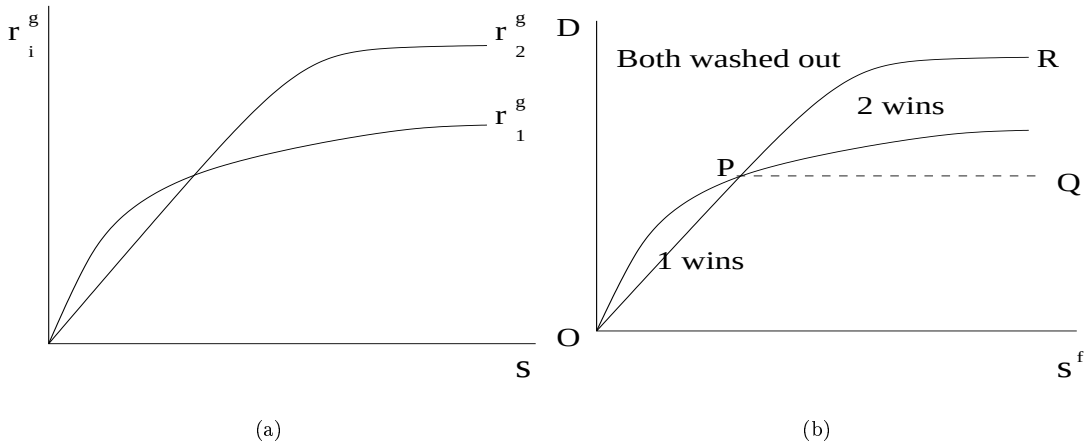


Figure 1: In environments containing a single growth-limiting substrate, the steady states of mixed-culture growth are completely determined by the single-species data. (a) Variation of the specific growth rates of the two species, denoted r_1^g and r_2^g, as a function of the substrate concentration, s. (b) The corresponding mixed-culture steady states at any dilution rate, D, and feed concentration, s^f. Only species 1 survives if D and s^f lie the region OPQ, and only species 2 survives if D and s^f are in the region QPR. Both species wash out for all other D and s^f.

biological ones are the least understood. By confining attention to growth in a chemostat, we deliberately minimize or eliminate the physicochemical processes (such as mixing), thus sharpening the focus on the biology of the system. Furthermore, the restriction to a chemostat ensures that the theory can be rigorously tested in a laboratory, which is a useful prelude to field experiments.

Intuition suggests that when multiple species engage in pure competion, it should be possible to predict their behavior *a priori* from single-species experiments. For, under these conditions, each species sees only the exogenous substrates in its environment — the other species are perceived through the effects they exert on the environment by consumption of the substrates. But the substrate consumption patterns of each species can be determined by performing single-species experiments. Thus, it seems plausible that given appropriate single-species data, one should be able to predict the behavior of mixed (multi-species) cultures.

The foregoing intuition is borne out when multiple species compete for a *single* growth-limiting substrate [1, 11, 12, 21, 27]. Under these conditions, the single-species data required to predict their behavior in mixed cultures consists of the *growth curves*, which define the variation of the specific growth rate, r_i^g, as a function of the growth-limiting substrate concentration, s (Figure 1a). Given these curves, we can predict the mixed-culture steady states at any dilution rate, D, and feed concentration, s^f. It suffices to generate Figure 1b from Figure 1a by replacing the s- and r_i^g-axes in Figure 1a with s^f and D, respectively, and drawing a horizontal line passing through the intersection point, if any, of the two growth curves. Figure 1b predicts that if the feed concentration is held fixed at a sufficiently high value, and the dilution rate is increased, only species 1 survives at low dilution rates, only species 2 survives at intermediate dilution rates, and both species wash out at high dilution rates [8]. This prediction has been confirmed by numerous experiments (reviewed in [9, Chapter 3] and [26]).

It is of considerable interest to determine if single-species data is also sufficient for predicting the behavior of mixed cultures limited by *multiple* growth-limiting substrates. A convenient starting point is the problem of mixed-culture growth in the presence of two growth-limiting substrates. This question has been addressed by a considerable body of work (reviewed in [9, Chapter 2]). The crux of these theoretical developments is that in two-substrate environments, no more than two species can coexist at steady state. Moreover, the existence and stability of the coexistence steady state at any given dilution rate and feed concentrations can be predicted *a priori* if we know two pieces of information derived from single-species data, namely [24, 25]

1. The *growth isocline* for each species, which is the locus of all substrate concentrations at which the

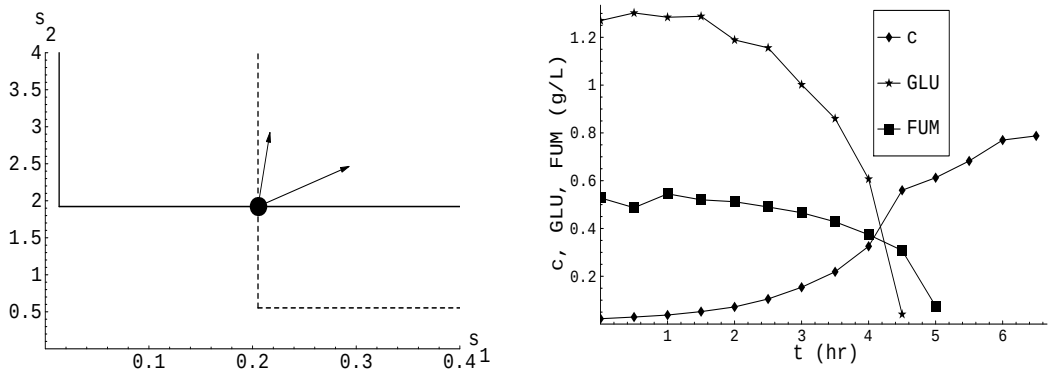


Figure 2: (a) Growth of *A. formosa* (species 1) and *C. meneghianiana* (species 2) on a mixture of phosphate (s_1) and silicate (s_2) [24, 25]. The full and dashed lines show the *growth isoclines* for species 1 and 2 at $D = 0.25\ 1/\text{day}$. They intersect at substrate concentrations representing a potential coexistence steady state. The arrows show the corresponding *consumption vectors,* (r^s_{11}, r^s_{12}) and (r^s_{21}, r^s_{22}), for the two species, where r^s_{ij} is the specific uptake rate of the j^{th} substrate by the i^{th} species. The growth isoclines and consumption vectors were calculated by assuming that r^g_i follows Liebig's law, and $r^s_{ij} = r^g_i/Y_{ij}$, where Y_{ij} denotes the single-substrate specific growth rate and yield of the i^{th} species on S_j. (b) Diauxic growth of *E. coli* K12 on a mixture of glucose and fumarate (from [19]). Consumption of fumarate does not begin until almost complete exhaustion of glucose.

species can maintain a specific growth rate equal to the given dilution rate. These curves determine the feasibility of coexistence steady states during mixed-culture growth — such steady states exist only if the growth isoclines for the two species intersect.

2. The two *consumption vectors* at the coexistence steady state(s), which represent the substrate uptake rates of the two species. These vectors determine whether the coexistence steady states are experimentally observable. More precisely, a coexistence steady state is stable only if the feed concentrations lie in the cone generated by the consumption vectors.

In the literature, the growth isoclines and consumption vectors are determined by making an additional hypothesis. Specifically, it is assumed that the growth and substrate uptake rates during mixed-substrate growth are completely determined by the growth and substrate uptake rates during *single-substrate* growth. This assumption works quite well if the two growth-limiting substrates are *essential* or *heterologous* (satisfy distinct nutritional requirements). In this case, the growth of both species is limited by a single substrate at all but a thin "band" of substrate concentrations. This thin band is often idealized as a curve by assuming that growth follows Liebig's law, $r^g_i = \min\{r^g_{i1}(s_1), r^g_{i2}(s_2)\}$, where $r^g_{ij}(s_j)$ denotes the single-substrate specific growth rate of the i^{th} species on S_j. Consequently, the growth isoclines and consumption vectors can be determined by the single-substrate data (Figure 2a). Many predictions of the theory for essential substrates have been verified (see [18] for a recent review).

The very same assumption — single-substrate data determines the mixed-substrate behavior — is also made when the growth-limiting substrates are *substitutable* or *homologous*, i.e., satisfy identical nutritional requirements (Figure 2b). More precisely, it is assumed that the specific growth rate of the i^{th} species, r^g_i, has the form

$$r^g_i(s_1, s_2) = r^g_{i1}(s_1) + r^g_{i2}(s_2) \tag{1}$$

where $r^g_{i1}(s_1)$ and $r^g_{i2}(s_2)$ represent the *single-substrate* specific growth rates of the i^{th} species on S_1 and S_2, respectively, and are often assumed to obey Monod kinetics [22]. But this assumption is not consistent with the experimental data for microbial growth on mixtures of substitutable substrates. Indeed

1. During batch growth on such mixtures, when the exogenous concentrations of both substrates are relatively high, one frequently observes *diauxic* growth [4, 14]. During such growth, the microbes

preferentially consume only one of the substrates, and it is only after exhaustion of this "preferred" substrate that they start consuming the other "less preferred" substrate (Figure 2b). Thus, the specific growth rate observed in such mixed-substrate experiments is identical to the maximum specific growth on the "preferred" substrate. In contrast, assumption (1) implies that the mixed-substrate specific growth rate equals the sum of the maximum specific growth rates on each of the substrates.

2. During mixed-substrate growth in continuous cultures, the cells stop consuming the "less preferred" substrate at a sufficiently high dilution rate (in Figure 5a, for example, there is no consumption of methanol for $D > 0.35$ 1/hr). The model predicts that this dilution rate is equal to the maximum specific growth rate on the "less preferred" substrate. But experiments show that this dilution rate is always larger than the maximum specific growth on the "less preferred" substrate [4].[1]

These discrepancies between the model and experiments arise because assumption (1) ignores the fact that the growth rate supported by a substrate is inhibited by the other substrate. Indeed, Monod and coworkers showed that diauxic growth occurs because one of the substrates completely blocks the uptake, and hence the growth, on the other substrate. Thus, a more accurate representation of the specific growth must have the form

$$r_i^g(s_1, s_2) = r_{i1}^g(s_1, s_2) + r_{i2}^g(s_1, s_2),$$

where $r_{i1}^g(s_1, s_2)$ and $r_{i2}^g(s_1, s_2)$ are decreasing functions of s_2 and s_1, respectively. Since these inhibitory effects are manifested only in the presence of *both* substrates, it is impossible to infer mixed-substrate behavior from single-substrate experiments, except at low growth rates or dilution rates when the inhibitory effects are negligibly small. Hence, any theory of mixed-culture growth on substitutable substrates must be developed without invoking assumption (1).

In this work, we make no attempt to infer mixed-substrate kinetics from single-substrate data. Instead, we show that the growth isoclines and the consumption vectors can be calculated directly from single-species, mixed-substrate experiments. Thus, we develop a complete theory that appeals only to the data — it makes no assumptions about the relationship between single- and mixed-substrate behavior. We show furthermore that in addition to the growth isoclines, it is useful to consider the *consumption curves*, i.e., the locus of substrate concentrations at which the rates of substrate supply and consumption are perfectly balanced. Consideration of the consumption curves along with the growth isoclines yields deeper insights into the steady states of mixed cultures. Specifically, we obtain

1. *A priori* bounds on the substrate concentrations that can be attained in mixed cultures.

2. Information about the stability of the steady states even if the growth isoclines are non-monotonic.

3. Additional insight into the nature of the transitions (bifurcations) that occur when the feed concentrations are changed at fixed dilution rate (or the dilution rate is changed at fixed feed concentrations).

Fortunately, the determination of consumption curves requires no additional effort. Given the growth isoclines and consumption vectors calculated from single-species experiments, the consumption curves can be determined without further experiments. Thus, the benefits of consumption curves can be realized at no further cost in terms of experiments.

The theory developed in this work rests upon three assumptions — pure competition, mutual inhibition of substrate uptake rates, and constant yields. There is substantial experimental evidence supporting the last two assumptions. These have been comprehensively reviewed by Egli and coworkers [4, 14], and we refer the reader to the references cited in these reviews for experimental tests of these assumptions. Here, we focus on specific experiments aimed at testing the validity of the first hypothesis, namely, pure competition. Most microbes excrete metabolic products, particularly at high dilution rates [10]. It is important to ensure that the system in question displays pure competition despite the release of excretory products.

Thus, the theory presented here achieves three goals

[1] In Tilman's terminology, the substrates are *perfectly substitutable* resources at low dilution rates, and *switching* resources at high dilution rates. However, the dilution rate at which the substrates undergo this transition is always greater than the washout dilution rate for the "less preferred" substrate.

1. It shows that the specific assumptions made in order to infer mixed-substrate behavior from single-substrate data are unnecessary. The single-species, mixed-substrate data is sufficient for predicting all features of mixed-culture growth.

2. It extends the resource-based approach by revealing the additional information embedded in the consumption curves.

3. It suggests experiments aimed at testing the hypotheses of the theory.

The paper is organized as follows. In Section 2, we describe the model, define the growth isoclines and the consumption curves, and show that they determine the properties of all the mixed-culture steady states. In Section 3, we describe experiments that may be used to test the model hypotheses and predictions. Finally, we summarize the conclusions in Section 4.

2 Results

2.1 The model

In a chemostat fed with sterile nutrients, the mass balances for the two growth-limiting substrates and species yield the equations [2, 17, 24]

$$\frac{ds_j}{dt} = D(s_j^f - s_j) - c_1 r_{1j}^s - c_2 r_{2j}^s, \quad j = 1, 2, \tag{2}$$

$$\frac{dc_i}{dt} = (r_i^g - D)\, c_i, \quad i = 1, 2, \tag{3}$$

where c_i (gdw/L) and s_j (g/L) denote the concentrations in the chemostat of the i^{th} species and the j^{th} substrate; D (1/hr) denotes the dilution rate; s_j^f (g/L) denotes the feed concentration of the j^{th} substrate; r_i^g (1/hr) denotes the specific growth rate of the i^{th} species; and r_{ij}^s (g/gdw-hr) denotes the specific uptake rate of the j^{th} substrate by the i^{th} species. To complete the model, we must specify the kinetics of growth (r_i^g) and substrate uptake (r_{ij}^s).

We assume that the specific growth and substrate uptake rates are functions of the exogenous substrate concentrations only, i.e.,

$$r_i^g(s_1, s_2),\ r_{ij}^s(s_1, s_2). \tag{4}$$

This is the mathematical representation of the assumption that the competition is pure, i.e., the growth and substrate uptake rates are independent of the cell densities.

Insofar as the specific uptake rates are concerned, we assume that there is no substrate uptake in the absence of the substrate, i.e.,

$$r_{i1}^s(0, s_2) = r_{i2}^s(s_1, 0) = 0, \tag{5}$$

and each substrate stimulates its own uptake

$$\frac{\partial r_{i1}^s}{\partial s_1}(s_1, s_2) > 0, \frac{\partial r_{i2}^s}{\partial s_2}(s_1, s_2) > 0. \tag{6}$$

Thus, we preclude the self-inhibitory effects often observed at relatively high substrate concentrations. We assume furthermore that each substrate inhibits the uptake of the other substrate, i.e.,

$$\frac{\partial r_{i1}^s}{\partial s_2}(s_1, s_2) < 0, \frac{\partial r_{i2}^s}{\partial s_1}(s_1, s_2) < 0. \tag{7}$$

This *mutual inhibition* is characteristic of growth on mixtures of substitutable substrates. It should be emphasized this assumption does not imply that each substrate directly inhibits the uptake of the other substrate. In reality, each substrate inhibits the synthesis of the transport enzymes for the other substrate [23]. However, since these enzymes are not variables of the model, (7) captures the net effect of the mutual inhibition.

It remains to specify the properties of the specific growth rates. To this end, let Y_{ij} denote the yield of the i^{th} species on S_j. Since the substrates are substitutable, the specific growth rate has the form

$$r_i^g(s_1, s_2) = Y_{i1} r_{i1}^s(s_1, s_2) + Y_{i2} r_{i2}^s(s_1, s_2) \tag{8}$$

We assume that the yields are constant.

The relations (4–8) embody all the assumptions required for the development of our theory. However, in previous work, it has often been assumed that $\partial r_i^g/\partial s_1, \partial r_i^g/\partial s_2 > 0$. Thus, it seems to appropriate to understand the conditions under which this additional assumption is manifested by our model. Now, (8) implies that

$$\frac{\partial r_i^g}{\partial s_1} = Y_{i1}\frac{\partial r_{i1}^s}{\partial s_1} + Y_{i2}\frac{\partial r_{i2}^s}{\partial s_1},\ \frac{\partial r_i^g}{\partial s_2} = Y_{i1}\frac{\partial r_{i1}^s}{\partial s_2} + Y_{i2}\frac{\partial r_{i2}^s}{\partial s_2}.$$

In general, nothing can be said about the signs of $\partial r_i^g/\partial s_1$ and $\partial r_i^g/\partial s_2$ because even though each substrate stimulates growth by promoting its own uptake, it also inhibits growth by depressing the uptake of the other substrate. For instance, the sign of $\partial r_i^g/\partial s_1$ is indeterminate because $\partial r_{i1}^s/\partial s_1 > 0$ (S_1 promotes its own uptake), but $\partial r_{i2}^s/\partial s_1 < 0$ (S_1 inhibits the uptake of S_2). It is only when the intensity of mutual inhibition, represented by the magnitudes of $\partial r_{i1}^s/\partial s_2$ and $\partial r_{i2}^s/\partial s_1$, is sufficiently small that both substrates stimulate the specific growth rate, i.e.,

$$\frac{\partial r_i^g}{\partial s_1}(s_1, s_2) > 0, \frac{\partial r_i^g}{\partial s_2}(s_1, s_2) > 0. \tag{9}$$

Accordingly, we shall refer to this as the case of *weak* mutual inhibition. While this is true in many instances, we show below that there are examples of mixed-substrate growth in which the specific growth rate is a decreasing function of one of the substrate concentrations. In what follows, we begin by assuming weak mutual inhibition. Later on, however, we shall relax this assumption.

2.2 There are three types of steady states

It follows from equations (2–3) that the model has 3 types of steady states

1. The *trivial* steady state at which neither species survives in the chemostat ($c_1 = c_2 = 0$). We denote it by ϕ_{00}.

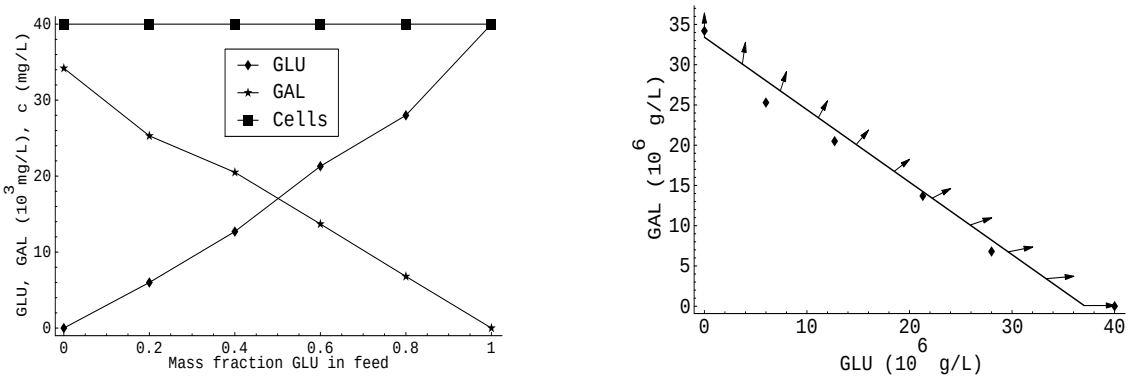
2. The *semitrivial* steady state at which only one of the species survives in the chemostat. There are two types of semitrivial steady states, $c_1 > 0, c_2 = 0$ and $c_1 = 0, c_2 > 0$. We denote them by ϕ_{10} and ϕ_{01}, respectively.

3. The *nontrivial* steady state at which both species coexist, i.e., $c_1 > 0, c_2 > 0$. We denote such steady states by ϕ_{11}.

Our goal is to show that given the validity of the model described above, the steady state data obtained from single-species experiments is sufficient for predicting the properties of the mixed-culture steady states.

2.3 Definition of the growth isoclines and consumption curves

We begin by defining the single-species data required to infer the properties of the mixed-culture steady states. To this end, we show first of all that the determination of the growth isoclines and consumption vectors from single-species experiments is, in effect, a method for characterizing the growth and substrate consumption kinetics of a given species. We then show that the determination of consumption curves and consumption vectors from single-species experiments is another way of obtaining the same information. Finally, we establish the equivalence of the two methods by showing that given the growth isoclines and consumption vectors, we can infer the consumption curves; conversely, the growth isoclines can be inferred from the consumption curves. Thus, either one of the two methods can be used to obtain the growth isoclines, consumption vectors, and consumption curves.



(a) (b)

Figure 3: Construction of the growth isoclines: (a) The steady state substrate concentrations and cell densities during growth of *E. coli* ML308 on a mixture of glucose and galactose at $D = 0.3$ 1/hr and varying feed concentrations (from [16]). The feed concentrations were varied by fixing the total sugar concentration at 100 mg/L and altering the composition. (b) The corresponding growth isocline and the associated specific substrate consumption vectors.

The growth isoclines and consumption vectors capture the growth and substrate consumption kinetics The growth isocline of the i^{th} species at dilution rate, D, denoted $\Upsilon_i(D)$, is the locus of all steady state substrate concentrations obtained when this species alone is grown on a mixture of S_1 and S_2 at the *fixed dilution rate, D*, and varying feed concentrations.

The upper panel of Figure 3 illustrates the construction of the growth isocline from single-species data. Figure 3a shows the steady state sugar concentrations obtained during growth of of *E. coli* ML308 on a mixture of glucose and galactose when the feed composition is changed at fixed dilution rate ($D = 0.3$ 1/hr) and total feed concentration ($s_1^f + s_2^f = 100$ mg/L). The growth isocline at $D = 0.3$ 1/hr is obtained from this data by plotting the pairs of glucose and galactose concentrations at various feed compositions on the plane of substrate concentrations (Figure 3b). Evidently, the same procedure can be performed at various dilution rates, thus generating a family of growth isoclines parametrized by the dilution rate.

Having determined the family of growth isoclines, one can draw vectors perpendicular to the growth isoclines, and pointing in the direction of increasing D. The magnitude of these vectors is arbitrary, but their direction is well-defined. For reasons given below, we shall refer to these vectors as the *growth limitation vectors*.

To understand the biological meaning of the experimentally determined growth isoclines and growth limitation vectors, it is useful to derive their mathematical counterparts in terms of the model. To this end, observe that the steady states for single-species growth of the i^{th} species satisfy the equations

$$D(s_1^f - s_1) = c_i r_{i1}^s(s_1, s_2), \tag{10}$$

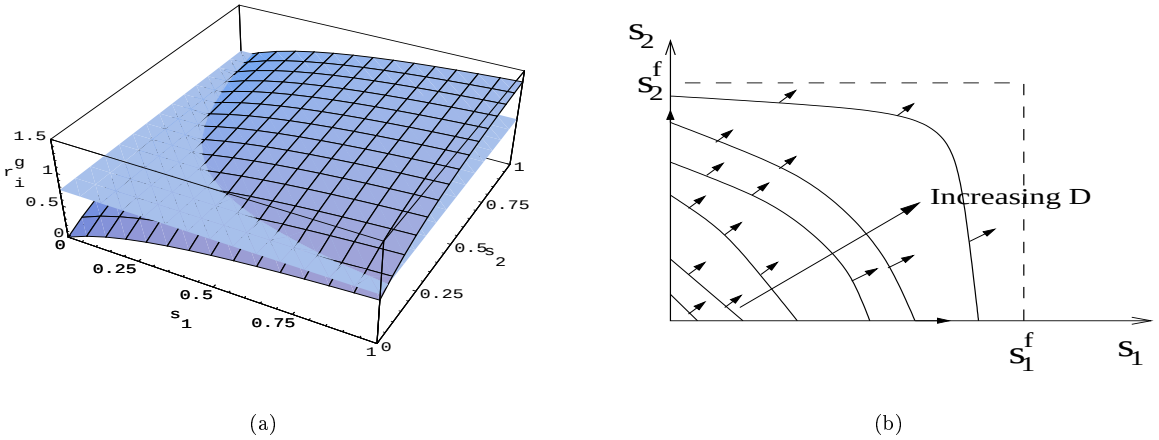
$$D(s_2^f - s_2) = c_i r_{i2}^s(s_1, s_2), \tag{11}$$

$$r_i^g(s_1, s_2) = D. \tag{12}$$

Evidently, equation (12) defines the growth isocline, since it determines the locus of all steady state concentrations attained at fixed D. Thus, the growth isocline for the i^{th} species at dilution rate, D, is the locus of all substrate concentrations at which the specific growth rate of the i^{th} species equals the dilution rate.

Since the growth isocline is given by (12), it is clear that the growth limitation vector defined above is parallel to the gradient of the specific growth rate

$$\nabla r_i^g(s_1, s_2) \equiv \left(\frac{\partial r_i^g}{\partial s_1}, \frac{\partial r_i^g}{\partial s_2} \right). \tag{13}$$



(a) (b)

Figure 4: Geometry of the growth isoclines: (a) The figure shows the typical surface of $r_i^g(s_1, s_2)$ in the case of weak mutual inhibition, when $r_i^g(s_1, s_2)$ is an increasing function of s_1 and s_2. Also shown in the figure is a plane of height, 0.75 1/hr, which intersects the surface of $r_i^g(s_1, s_2)$. The growth isocline of the i^{th} species at $D = 0.75$ 1/hr is the projection of this curve of intersection onto the s_1s_2-plane. It is clear from the figure that this growth isocline is a decreasing curve on the s_1s_2-plane. At substrate concentrations above the growth isocline, $r_i^g(s_1, s_2) > 0.75$ 1/hr; at substrate concentrations below the growth isocline, $r_i^g(s_1, s_2) < 0.75$ 1/hr. Moreover, the higher the value of D, the farther the corresponding growth isocline from the origin of the s_1s_2-plane. (b) Consequently, the growth isoclines at various D form a family of decreasing curves (shown as full lines). The dashed lines show a hypothetical path along which the feed concentrations are changed in order to generate the growth isoclines.

It follows that the slope of the growth limitation vector provides a measure of the extent to which growth is limited by S_1 or S_2. Indeed, if the slope of this vector is zero, then so is the slope of $\nabla r_i^g(\mathbf{s})$. In this case, (13) implies that $\partial r_i^g/\partial s_2 = 0$, i.e., growth is limited exclusively by S_1. More generally, if the slope of the growth limitation vector is small but not zero, we may say that growth is more limited by S_1 rather than S_2. Similarly, if the slope of the growth limitation vector is large, growth is more limited by S_2 rather than S_1.

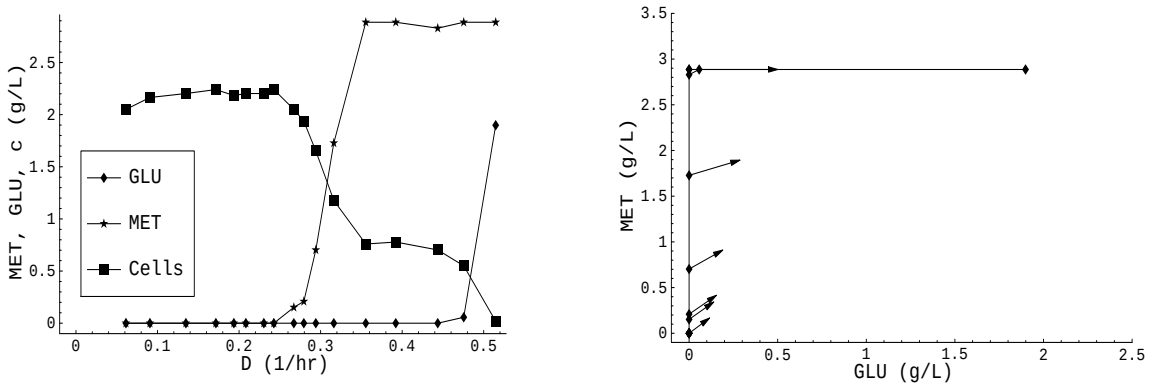
The shape of the growth isoclines reflects the geometry of the surface of $r_i^g(s_1, s_2)$. If the mutual inhibition is weak, the specific growth rate is an increasing function of both substrate concentrations, so that the growth isocline at any D is a decreasing curve (Figure 4). At substrate concentrations below the growth isocline, the specific growth rate is less than the dilution rate ($r_i^g < D$); at substrate concentrations above the growth isocline, it exceeds the dilution rate ($r_i^g > D$). Moreover, the higher the value of D, the further the distance of the growth isocline from the origin.

Thus, the construction of growth isoclines from single-species experiments reveals the behavior of the function, $r_i^g(s_1, s_2)$. But the single-species experiments also yield the specific substrate uptake rates. Indeed, (10–11) imply that

$$r_{i1}^s(s_1, s_2) = \frac{D(s_1^f - s_1)}{c_i},\ r_{i2}^s(s_1, s_2) = \frac{D(s_2^f - s_2)}{c_i}. \tag{14}$$

Since the operating conditions (D, s_1^f, s_2^f) are known, and the steady state concentrations (s_1, s_2, c_i) are measured, we can calculate $r_{i1}^s(s_1, s_2)$ and $r_{i2}^s(s_1, s_2)$ at every pair of substrate concentrations used to construct the growth isocline. It is convenient to represent the two specific uptake rates by a vector, $\mathbf{r_i^s}(\mathbf{s}) \equiv (r_{i1}^s(\mathbf{s}), r_{i2}^s(\mathbf{s}))$, based at the corresponding steady state substrate concentrations (see Figure 3b). We shall refer to $\mathbf{r_i^s}(\mathbf{s})$ as the *specific substrate consumption vector* of the i^{th} species.

The slope of $\mathbf{r_i^s}(\mathbf{s})$ reveals the substrate consumption behavior of the i^{th} species. If the slope of $\mathbf{r_i^s}(\mathbf{s})$ is small, then $r_{i2}^s(\mathbf{s}) \ll r_{i1}^s(\mathbf{s})$, which means that the i^{th} species consumes more S_1 rather than S_2. If the slope is large, it consumes more S_2 rather than S_1.



(a) (b)

Figure 5: Construction of the consumption curves: (a) The steady state substrate concentrations during growth of *H. polymorpha* on a mixture of glucose and methanol at fixed feed concentrations and varying D (from [5]). (b) The corresponding consumption curve and associated specific substrate consumption vectors.

The generation of Figure 4b from single species data provides complete information about the kinetics of growth and substrate consumption. Given any (s_1, s_2) on a growth isocline, the corresponding specific growth rate is equal to the dilution rate at which the growth isocline was constructed, and the specific substrate uptake rates are given by the components of the specific substrate consumption vector at (s_1, s_2). Thus, the construction of growth isoclines and associated specific consumption vectors from single-species data is essentially a method for characterizing the behavior of the specific growth rate, $r_i^g(s_1, s_2)$, and the specific substrate uptake rates, $r_{i1}^s(s_1, s_2), r_{i2}^s(s_1, s_2)$. By fixing the dilution rate in these experiments, we are choosing particular paths on the s_1s_2-plane — namely, the growth isoclines at various D — along which the specific growth and substrate uptake rates are determined.

Now, there is nothing special about the paths corresponding to the growth isoclines. We could just as well measure the specific growth and consumption rates along any other path on the s_1s_2-plane. The consumption curves described below represent another set of paths for sampling the specific growth and substrate consumption rates in single-species experiments.

The consumption curves and consumption vectors also capture the growth and substrate uptake kinetics The consumption curve of the i^{th} species at feed concentrations, s_1^f, s_2^f, denoted $\Phi_i(\mathbf{s^f})$, is the locus of all steady state substrate concentrations obtained when this species alone is grown on a mixture of S_1 and S_2 at *fixed feed concentrations*, s_1^f, s_2^f, but varying dilution rates.

The upper panel of Figure 5 illustrates the construction of the consumption curve from single-species data. Figure 5a shows the steady state glucose and methanol concentrations obtained when *H. polymorpha* is grown on a mixture of glucose and methanol at fixed feed concentrations and varying dilution rates. The corresponding consumption curve is obtained by plotting the glucose and methanol concentrations at various dilution rates on the plane of substrate concentrations (Figure 5b). By appealing to (14), we can also calculate the specific substrate uptake rates at each point of the consumption curve, and represent them graphically as specific substrate consumption vectors based at corresponding substrate concentrations (Figure 5b).

We gain insight into the biological significance of the consumption curve by deriving its equation in terms of the model. To this end, we observe that the steady states for single-species growth of the i^{th} species must satisfy equations (10–11). These equations define the consumption curve, since eliminating D from these equations yields the relation

$$\frac{s_2^f - s_2}{s_1^f - s_1} = \frac{r_{i2}^s(s_1, s_2)}{r_{i1}^s(s_1, s_2)}, \tag{15}$$

which defines the locus of steady state substrate concentrations attained at the fixed feed concentrations, s_1^f and s_2^f. It follows from the defining equations (10–11) that the consumption curve for the i^{th} species is the locus of substrate concentrations at which the rates of supply of both substrates are equal their consumption rates.

It is useful to obtain a more concise characterization of the consumption curve by appealing to (15) rather than the defining equations, (10–11). To this end, define the *substrate depletion vector*, $\triangle(\mathbf{s}) \equiv (s_1^f - s_1, s_2^f - s_2)$, at each pair of substrate concentrations. Then, (15) says that the slopes of $\mathbf{r_i^s}(\mathbf{s})$ and $\triangle(\mathbf{s})$ are equal at every point of a consumption curve. Hence, the consumption curve can also be viewed as the locus of all substrate concentrations at which $\mathbf{r_i^s}(\mathbf{s})$ is parallel to $\triangle(\mathbf{s})$. This property of consumption curves is evident in Figure 5b.

At substrate concentrations above the consumption curve, the slope of $\mathbf{r_i^s}(\mathbf{s})$ is higher than the slope of $\triangle(\mathbf{s})$ (Figure 6a). To see this, observe that at a point O on the consumption curve, $\mathbf{r_i^s}(\mathbf{s})$ is parallel to $\triangle(\mathbf{s})$. Now, suppose we move from O to P, so that s_1 is constant and s_2 increases. Then the slope of $\triangle(\mathbf{s}) \equiv (s_1^f - s_1, s_2^f - s_2)$ decreases and the slope of $\mathbf{r_i^s}(\mathbf{s}) \equiv (r_{i1}^s(s_1, s_2), r_{i2}^s(s_1, s_2))$ increases (because r_{i1}^s is a decreasing function of s_2, and r_{i2}^s is an increasing function of s_2). It follows that at P, the slope of $\mathbf{r_i^s}(\mathbf{s})$ is higher than the slope of $\triangle(\mathbf{s})$. If we move from O to Q (Figure 6a), a similar argument shows that at substrate concentrations below the consumption curve, the slope of $\mathbf{r_i^s}(\mathbf{s})$ is lower than the slope of $\triangle(\mathbf{s})$.

The foregoing fact immediately imply the following important result. Given any feed concentrations, $\mathbf{s^f}$, and the corresponding consumption curves, $\Phi_1(\mathbf{s^f})$, $\Phi_2(\mathbf{s^f})$, for the two species, $\triangle(\mathbf{s})$ lies between $\mathbf{r_i^s}(\mathbf{s})$ and $\mathbf{r_i^s}(\mathbf{s})$ precisely when the substrate concentrations lie between the two consumption curves (Figures 6b,c). We shall appeal to this result later.

Figure 5b shows that as D increases from near-zero values to the washout dilution rate, the consumption curve traverses an increasing path on the s_1s_2-plane that starts at the origin, $(s_1 = s_2 = 0)$, and terminates at the feed point, $s_1 = s_1^f, s_2 = s_2^f$. The same trend is predicted by the model. Indeed, since the consumption curve satisfies the equation, $r_{i1}^s(s_1, s_2)(s_2^f - s_2) - r_{i2}^s(s_1, s_2)(s_1^f - s_1) = 0$, it is clear that origin and the feed point lie on the consumption curve. To see why the model predicts the increasing trend, note that $\mathbf{r_i^s}(\mathbf{s})$ and $\triangle(\mathbf{s})$ must be parallel along a consumption curve. This implies that as we move along a consumption curve, both vectors must turn the same way (clockwise or counterclockwise). However, one can check that along non-increasing curves, $\mathbf{r_i^s}(\mathbf{s})$ and $\triangle(\mathbf{s})$ turn in opposite directions. It follows that consumption curves must be increasing.

Figure 6d shows a hypothetical family of consumption curves generated from single-species data. Just like the family of growth isoclines, the family of consumption curves give us complete information about the kinetics of growth and substrate consumption. Indeed, each point on a consumption curve corresponds to some dilution rate. Thus, given any point on the consumption curve, the corresponding specific growth rate is equal to the dilution rate at that point. The specific substrate uptake rates are given by the specific substrate consumption vectors at that point. It is therefore clear that Figures 4b and 6d contain the same information. They merely represent two different sets of paths for capturing this information.

2.3.1 The two methods for characterizing the growth and substrate consumption kinetics are equivalent

The growth isoclines can be inferred from Figure 6d. To generate the growth isocline at any given D, it suffices to join the points on distinct consumption curves corresponding to this D. Conversely, given the feed concentrations, we can generate the corresponding consumption curve from Figure 4b. For this, it suffices to scan each growth isocline until we find the point $\mathbf{s}$ at which $\mathbf{r_i^s}(\mathbf{s})$ and $\triangle(\mathbf{s})$ are parallel. Having determined such a point on each growth isocline, we can join these points to generate the consumption curve.

Thus, we can perform single-species experiments to generate Figure 4b, and infer the consumption curves from it. Alternatively, we can perform single-species experiments to generate Figure 6d and infer the growth isoclines. Both methods give us complete information about the growth and substrate consumption kinetics of a given species (although the latter approach seems easier than the former since it involves nothing more than joining points corresponding to the same D).

2.4 Properties of mixed-culture steady states

In what follows, it is assumed that by appealing to one of the two methods described above, we have generated the growth isoclines and consumption curves for both species at some dilution rate D and feed concentrations, $\mathbf{s^f}$. Our goal is to show that the existence and stability of the mixed-culture steady states under these conditions are completely determined by these curves. These results have already been derived in terms of growth isoclines and consumption vectors [17, 25, 24]. Our goal is to show that the consumption curves yield further insights into semitrivial and nontrivial steady states.[2]

Trivial steady state At ϕ_{00}, there are no cells in the reactor ($c_1 = c_2 = 0$) and the substrate concentrations are equal to the feed concentrations. Thus, on the s_1s_2-plane, this steady state coincides with the feed point, $\mathbf{s^f}$.

The trivial steady state always exists — one can always arrange to have a sterile chemostat at any dilution rate and feed concentrations. However, from the mathematical point of view, this steady state is not considered stable unless sterility is maintained even if the chemostat is inoculated with both species. It is shown in Appendix A that the trivial steady state is stable precisely when $D > r_1^g(\mathbf{s^f}), r_2^g(\mathbf{s^f})$, i.e., D exceeds the maximum specific growth rates consistent with the feed concentrations. At lower dilution rates, at least one of the species will succeed in establishing itself in the chemostat.

In the case of weak mutual inhibition, the stability of the trivial steady can be inferred by inspection of the two growth isoclines. The trivial steady state is stable precisely when the feed concentrations lie in the region OPQR lying below both growth isoclines (Figure 7a). To see this, it suffices to recall that in the case of weak inhibition, $r_i^g(\mathbf{s}) < D$ for all $\mathbf{s}$ lying below the growth isocline for the i^{th} species. Hence, $r_1^g(\mathbf{s_f}), r_2^g(\mathbf{s_f}) < D$ for all $\mathbf{s^f}$ lying below both growth isoclines.

Semitrivial steady states We confine our attention to the semitrivial steady state, ϕ_{10}, at which only species 1 thrives in the chemostat ($c_1 > 0, c_2 = 0$). The results for ϕ_{01} are analogous.

Given any D and $\mathbf{s^f}$, the steady state substrate concentrations at the corresponding semitrivial steady state(s) lie at the intersection of the growth isocline, $\Upsilon_1(D)$, and the consumption curve, $\Phi_1(\mathbf{s_f})$ (Figure 7b). To see this, recall that

1. The growth isocline, $\Upsilon_1(D)$, is the locus of all steady state substrate concentrations attained when species 1 alone grows at the dilution rate, D.

2. The consumption curve, $\Phi_1(\mathbf{s_f})$, is the locus of all steady state substrate concentrations attained when species 1 alone grows at the feed concentrations, $\mathbf{s^f}$.

Hence, the steady state substrate concentrations during growth of species 1 alone at the dilution rate, D, and feed concentrations, $\mathbf{s^f}$, are given by the intersection point(s) of $\Upsilon_1(D)$ and $\Phi_1(\mathbf{s^f})$. It follows that this steady state exists only if the feed concentrations lie above the growth isocline. In the case of weak inhibition, there is at most one such steady state, since $\Phi_1(\mathbf{s^f})$ is an increasing curve, and $\Upsilon_1(D)$ is a decreasing curve (Figure 4).

The mere existence of the semitrivial steady state, ϕ_{10}, does not imply that it will be observed in mixed-culture experiments. It is experimentally observable if it is stable with respect to small perturbations of the substrate concentrations and cell densities. It is shown in Appendix A that ϕ_{10} is stable provided

1. $\nabla r_1^g|_{\phi_{10}} \cdot \mathbf{t}_1|_{\phi_{10}} > 0$, where $\mathbf{t}_1|_{\phi_{10}}$ denotes the tangent to the consumption curve for species 1 at the substrate concentrations corresponding to ϕ_{10}, and oriented in the direction of increasing substrate concentrations.

2. $r_2^g|_{\phi_{10}} < D = r_1^g|_{\phi_{10}}$, where $r_i^g|_{\phi_{10}}$ denotes the specific growth rate of the i^{th} species at the steady state, ϕ_{10}.

[2] Although the consumption curves provide no new information about the trivial steady state, we have included a brief discussion of this steady state for the sake of completeness.

These stability conditions have simple physical interpretations. The first condition says that if the substrate concentrations are increased ever so slightly along the consumption curve, the specific growth rate must increase. The increase in the specific growth rate serves to increase the cell density, thus negating the effect of the increased substrate concentrations, and restoring the chemostat to its original state.[3] The second condition says at ϕ_{10}, the specific growth rate of species 2 should be less than D, the specific growth rate at which species 1 is already growing in the chemostat. This condition ensures that if perturb the steady state by introducing a small inoculum of species 2 into the chemostat, the chemostat will return to its original state of single-species growth on species 1.

The growth isoclines and consumption curves immediately tell us whether these stability conditions will be satisfied in the mixed-culture experiment. We can easily check if the first condition is satisfied, since $\mathbf{t}_1$ is tangential to the consumption curve and ∇r_1^g is perpendicular to the growth isocline. In the case of weak mutual inhibition, the first condition is always satisfied ($\nabla r_1^g \cdot \mathbf{t}_1 > 0$), which reflects the fact that under these conditions, r_1^g is an increasing function of s_1 and s_2. The second condition may or may not be satisfied. To this end, recall that the $r_2^g < D$ precisely when the substrate concentrations lie below the growth isocline for species 2. Thus, ϕ_{10} is stable if the substrate concentrations at this steady state lie below $\Upsilon_2(D)$ (Figure 7b); it is unstable if the substrate concentrations at this steady state lie above $\Upsilon_2(D)$.

A similar argument for the other semitrivial steady state, ϕ_{01}, shows that the substrate concentrations at this steady state lie at the intersection of $\Upsilon_2(D)$ and $\Phi_2(\mathbf{s^f})$. This steady state is stable precisely when the corresponding substrate concentrations lie below the growth isocline, $\Upsilon_1(D)$.

Hence, given the curves, $\Upsilon_1(D)$, $\Phi_1(\mathbf{s^f})$, for species 1, and curves, $\Upsilon_2(D)$, $\Phi_2(\mathbf{s^f})$ for species 2, we can predict the existence and stability of ϕ_{10} and ϕ_{01} at D and $\mathbf{s^f}$.

Nontrivial steady state It is not particularly surprising that the the growth isoclines and consumption curves yield complete information about the existence and stability of the semitrivial steady state, since these steady states are identical to single-species steady states. However, it is remarkable that they reveal the properties of the nontrivial steady states, which have no counterpart in single-species cultures.

It turns out that the substrate concentrations at the nontrivial steady states are given by those intersection points of the two growth isoclines that lie inside the region enclosed by the two consumption curves. Thus, given the two growth isoclines, we can immediately identify the substrate concentrations at all the coexistence steady states.

To see why the nontrivial states can be obtained in this fashion, observe that a nontrivial steady state satisfies the equations

$$0 = D(s_j^f - s_j) - c_1 r_{1j}^s(s_1, s_2) - c_2 r_{2j}^s(s_1, s_2), \quad j = 1, 2, \tag{16}$$

$$0 = r_i^g(s_1, s_2) - D, \quad i = 1, 2, \tag{17}$$

It follows from (17) that the substrate concentrations at a nontrivial steady states lie at the intersection points of the two growth isoclines. However, all such substrate concentrations do not necessarily correspond to coexistence steady states. To see this, observe that the substrate concentrations must also satisfy equation (16), which can be rewritten in the vectorial form

$$c_1 \mathbf{r_1^s}(\mathbf{s}) + c_2 \mathbf{r_2^s}(\mathbf{s}) = D\triangle(\mathbf{s}). \tag{18}$$

This equation has positive solutions, $c_1, c_2 > 0$ precisely when $\triangle(\mathbf{s})$ lies between $\mathbf{r_1^s}(\mathbf{s})$ and $\mathbf{r_2^s}(\mathbf{s})$. The latter is true precisely when the substrate concentrations lie between the two consumption curves (see Figures 6b,c). Thus, the substrate concentrations at nontrivial steady states are given by only those intersection points of the growth isoclines that lie within the region enclosed by the two consumption curves.

Given particular feed concentrations, $\mathbf{s^f}$, the consumption curves for the two species delineate the substrate concentrations that can be attained at coexistence steady states. In other words, the coxistence steady

[3] This condition becomes more plausible if we observe that, in general, the substrate concentrations are self-stabilizing. If the substrate concentrations are increased slightly from their steady state values, $ds_j/dt < 0$ because Ds_j and $r_{1j}^s(s_1, s_2)$ increase. However, this is not the case when the substrate concentrations are perturbed slightly along the tangent to the consumption curve. The new substrate concentrations attained after such perturbations are, to a first degree of approximation, still on the consumption curve, so that $ds_j/dt = D(s_j^f - s_j) - r_{1j}^s(s_1, s_2)c_1 \approx 0$. Since the substrate concentrations are not self-stabilizing, the specific growth rate, and hence, the cell density must increase to restore the system to its original state.

state exists precisely when the two growth isoclines intersect within the region enclosed by the consumption curves. Thus, we have referred to the two consumption curves as the *envelope of coexistence* [20].

To determine whether the nontrivial steady state(s) are observable, it is necessary to resolve the question of their stability. It is shown in Appendix A that a nontrivial steady state is stable only if the two pairs of vectors, $(\mathbf{r_1^s}, \mathbf{r_2^s})$ and $(\nabla r_1^g, \nabla r_2^g)$ have the same *orientation*, i.e.,

1. Either $\mathbf{r_1^s}$ lies above $\mathbf{r_2^s}$ and ∇r_1^g lies above ∇r_2^g .
2. Or $\mathbf{r_1^s}$ lies below $\mathbf{r_2^s}$ and ∇r_1^g lies below ∇r_2^g.

This stability criterion says that for a coexistence steady state to be stable, it is necessary that each species consume more of the substrate that limits its growth more [17, 24]. In the first case, for instance, ∇r_1^g above ∇r_2^g means that growth of species 1 is more limited by S_2, and growth of species 2 is more limited by S_1. The condition, $\mathbf{r_1^s}$ above $\mathbf{r_2^s}$ says that species 1 consumes more S_2, and species 2 consumes more S_1. Thus, both species consume more of the substrate that more strongly limits their growth (S_2 for species 1, and S_1 for species 2). The second case lends itself to a similar interpretation.

The single-species data enables us to immediately test the validity of this criterion. Indeed, since the growth isoclines are known, we can find the orientations of ∇r_1^g and ∇r_2^g at a coexistence steady state — they are perpendicular to the growth isoclines at the coexistence steady state. Given the consumption curves, we can also infer the orientations of $\mathbf{r_1^s}$ and $\mathbf{r_2^s}$ at any coexistence steady state (see Figure 6b,c). In fact, since we know the consumption vectors at various points on both growth isoclines, we calculate $\mathbf{r_1^s}$ and $\mathbf{r_2^s}$ at any coexistence steady state. Given these vectors, we can predict the cell densities of both species at the coexistence steady state by solving equation (18).

2.5 Applications of the theory

In what follows, we apply the foregoing results to various hypothetical cases. The conclusions reached will form the basis for the experiments described in the next section.

2.5.1 Fixed dilution rate and feed concentrations

We begin by considering the implications of the theory when the dilution rate rate and feed concentrations are fixed. Specifically, we assume that the growth isoclines and consumption curves at some dilution rate, D, and feed concentrations, $\mathbf{s^f}$, have been constructed from single-species experiments. We show that we can the properties of all the mixed-culture steady states that would be obtained at this D and $\mathbf{s^f}$.

Figure 8 shows 4 hypothetical arrangements of the growth isoclines and consumption curves for the two species. We begin by the applying the results to the arrangement shown in Figure 8a. In this case

1. The semitrivial steady state, ϕ_{10}, lies at the intersection of the growth isocline and the consumption curve for species 1 ($\Upsilon_1(D)$ and $\Phi_1(\mathbf{s^f})$, respectively). It is unstable because it lies above $\Upsilon_2(D)$, the growth isocline for species 2.
2. The semitrivial steady state, ϕ_{01}, lies at the intersection of the growth isocline and the consumption curve for species 2 ($\Upsilon_2(D)$ and $\Phi_2(\mathbf{s^f})$, respectively). It is unstable because it lies above $\Upsilon_1(D)$, the growth isocline for species 1.
3. The nontrivial steady state, ϕ_{11}, lies at the intersection of the two growth isoclines. It is a legitimate nontrivial steady state because the corresponding substrate concentrations lie between the two consumption curves. It is likely to be stable since $\mathbf{r_1^s}$ lies below $\mathbf{r_2^s}$ and ∇r_1^g lies below ∇r_2^g.

If the growth isoclines and consumption curves have the disposition shown in Figure 8b

1. The semitrivial steady state, ϕ_{10}, which lies at the intersection of $\Upsilon_1(D)$ and $\Phi_1(\mathbf{s^f})$, is stable because it lies below $\Upsilon_2(D)$, the growth isocline for species 2.
2. The semitrivial steady state, ϕ_{01}, which lies at the intersection of $\Upsilon_2(D)$ and $\Phi_2(\mathbf{s^f})$, is stable because it lies below $\Upsilon_1(D)$, the growth isocline for species 1.

3. The nontrivial steady state, ϕ_{11}, which lies at the intersection of the two growth isoclines, is certainly unstable since $\mathbf{r_1^s}$ lies below $\mathbf{r_2^s}$, but ∇r_1^g lies above ∇r_2^g.

The remaining two cases in Figure 8 can be understood by similar arguments.

2.5.2 Fixed dilution rate and varying feed concentrations

Next, we consider the behavior in response to varying feed concentrations. Specifically, we assume that the chemostat is at a (stable) coexistence steady state at some dilution rate, D, and feed concentrations, $\mathbf{s^f}$, and we ask: How does the chemostat respond if the dilution is fixed, and the feed concentrations are changed.

Tilman obtained the complete answer to this question [24, 25]. Figure 9a shows the behavior that would be observed at all possible feed concentrations. Since the dilution rate is fixed, the growth isoclines, and hence, the substrate concentrations at the coexistence steady state remain unchanged. Tilman showed that

1. Both species coexist precisely when the feed concentrations lie in the cone, SQT, generated by the specific substrate consumption vectors at the coexistence steady state. This follows immediately from (18), which says that coexistence steady states exist precisely when $\triangle(\mathbf{s})$ lies between $\mathbf{r_1^s}$ and $\mathbf{r_2^s}$.

2. Both species wash out when the feed concentrations lie in the region, OPQR.

3. Only one of the species thrives when the feed concentration lie in the regions above PQS or below RQT.

It follows from this picture that if the ratio of the feed concentrations, s_2^f/s_1^f, is too high or too low, one of the species will be rendered extinct. This result, often referred to as the *resource-ratio hypothesis*, is an important consequence of the theory. It suggests an explanation for the *paradox of enrichment* — enrichment of an ecosystem with only one of nutrients reduces its biodiversity.

By appealing to consumption curves, we can see exactly why the coexistence steady state ceases to exist if the feed concentrations lie outside the cone, SQT. Figure 9b shows the manner in which the consumption curves are shifted when s_2^f is decreased at fixed s_1^f (the original and new consumption curves are shown as black and red curves, respectively). At sufficiently small s_2^f, the growth isoclines intersect outside the envelope of coexistence; hence, the coexistence steady state ceases to exist. Under these conditions, species 1 becomes dominant, since the other semitrivial steady state, ϕ_{01}, is unstable.

2.6 The case of strong mutual inhibition

The experimental data suggests that in some instances, the assumption of weak inhibition ($\partial r_i^g/\partial s_1, \partial r_i^g/\partial s_2 > 0$) is violated. For instance, the specific growth rates of *H. polymorpha* on glucose, xylose, and glycerol are 0.61, 0.175, and 0.27 1/hr, respectively [3]. During mixed-substrate growth on glucose + xylose and glucose + glycerol, the specific growth rates are 0.36 1/hr and 0.37 1/hr, respectively. Thus, addition of xylose or glycerol to a culture growing on glucose decreases the growth rate, whereas addition of glucose to a culture growing on xylose or glycerol increases the specific growth rate. Likewise, addition of 3-phenylpropionic acid to a culture of *E. coli* ML308 growing on glucose decreases the growth rate, whereas addition of glucose to a culture growing on 3-phenylpropionic acid increases the growth rate [13]. It follows that for certain substrate concentrations, $\partial r_i^g/\partial s_1, \partial r_i^g/\partial s_2$ have opposite signs, and the growth isoclines may not be monotonically decreasing.

When the growth isoclines are not monotonic, there may be multiple semitrivial steady states. Moreover, some of these steady states may be unstable because $\nabla r_1^g.\mathbf{t}_1$ is negative (a condition that is impossible in the case of weak inhibition). This instability arises because the specific growth rate is a decreasing function of the substrate concentrations along certain directions in the neighborhood of the steady state. Perturbations that increase the substrate concentrations along such directions decrease the specific growth rate, which destabilizes the system by reducing the cell density in the chemostat.

Thus, in the case of strong mutual inhibition, the consumption curves, which provide the vector, $\mathbf{t_1}$, are crucial for determining the stability of the semitrivial steady states.

3 Discussion

We have shown above that a complete theory of mixed-culture growth on substitutable substrates can be developed without making specific assumptions about the kinetic expressions. Since the theory appeals directly to the data, the assumptions required for predicting mixed-culture growth are relatively weak. We have also shown that the consumption curves can be inferred from the single-species data, and that they provide additional insights into the existence and stability of the mixed-culture steady states. In what follows, we describe experiments that may be designed to test the hypotheses and predictions of the theory.

3.1 Experiments for testing the validity of the model hypotheses

The first hypothesis (4) states that the specific growth and uptake rates depend only on the substrate concentrations — they are independent of the cell densities. If this hypothesis is true, then

1. Equations (12) and (14) imply that the growth isocline and the associated consumption vectors are completely determined by the identity of the species and the dilution rate — they are independent of the feed concentrations. For example, the growth isoclines and specific substrate consumption vectors shown in Figure 4b were generated by varying the feed composition at fixed $D = 0.3$ 1/hr and $s_1^f + s_2^f = 100$ mg/L. If the specific growth and consumption rates are truly independent of the cell density, the very same growth isocline and specific substrate consumption vectors would be obtained if the experiments were performed at the same D, but different total feed concentration (say, 200 mg/L).

2. Equation (17) implies that the steady state concentrations of a coexistence steady state are independent of the feed concentrations. To test this hypothesis, suppose we have a coexistence steady state at some dilution rate and feed concentrations. If our hypothesis is correct, the steady state substrate concentrations will not change even if we change the feed concentrations.

These experiments are analogous to those performed for verification of the Monod model for single-substrate, single-species growth. The Monod model predicts that the steady state substrate concentration depends only on the dilution rate — it is independent of the feed concentrations. Experiments have shown that this is indeed the case.

The second hypothesis is that each substrate promotes its own own uptake and inhibits the uptake of the other substrate (6–7). This hypothesis implies that the consumption curve is a strictly increasing. Thus, the experimental consumption curves provide an indirect test of this hypothesis. It can also be tested directly by transferring a sample of the steady state single-species culture in the chemostat to a shake flask, spiking the sample with a small amount of a substrate, and measuring the uptake rates of both substrates. Such experiments have been performed by Lendenmann & Egli [15].

The third and final hypothesis is that the yields are constant. An indirect test follows from the fact that if this hypothesis is true, the steady state cell density in single-species experiments is given by

$$c_i = Y_{i1}(s_1^f - s_1) + Y_{i2}(s_2^f - s_2)$$

where Y_{i1}, Y_{i2} are the yields of the i^{th} species during single-substrate growth on S_1 and S_2. If the observed cell densities do not agree with the cell densities calculated from this expression, the hypothesis cannot be true. Direct tests of this hypothesis can be performed with radioactively labeled substrates (see [6] for an example)

3.2 Experiments for testing the validity of the model predictions

Few predictions of resource-based theory have been tested rigorously (see [18] for a comprehensive review). Figures 8 and 9 illustrate some predictions of the theory. Specifically, the model predicts

1. The dilution rates and feed concentrations at which coexistence will be observed.

2. The substrate concentrations and cell densities attained at any coexistence state.

3. The manner in which the chemostat will respond if the feed concentrations are varied at a fixed dilution rate.

All these predictions can be tested experimentally. Insofar as substitutable substrates are concerned, the last prediction was tested by Rothhaupt with a system in which two rotifers competed for growth on two algal species [22]. However, none of these predictions have been tested in microbial systems.

4 Conclusions

We extended the theory of mixed-culture growth on mixtures of substrates in two directions. Specifically, we showed that

1. The single-species data completely determines the behavior of the mixed culture. It is not necessary to make specific assumptions about the kinetics of growth and substrate consumption. Relatively weak assumptions about the qualitative properties of growth and substrate consumption are sufficient for inferring the properties of the mixed culture.

2. There is nothing special about the growth isoclines. They represent a set of paths on the s_1s_2-plane along which one can acquire information about the growth and substrate consumption rates. The same information can be obtained by choosing another set of paths, namely, the consumption curves. Importantly, the consumption curves can be deduced from the growth isoclines and consumption vectors; conversely, the growth isoclines can be inferred from the consumption curves. The two sets of curves are therefore "dual" to each other.

3. Consideration of the growth isoclines together with the consumption curves yields deeper insights into the model. Specifically, we showed that the consumption curves delineate all possible substrate concentrations attained by coexistence steady state, enable us to define stability criteria for systems in which the growth isoclines are non-monotonic, and elucidate the bifurcations in response to varying dilution rates and feed concentrations..

Finally, we discussed experiments that can be used to test the validity of the model hypotheses and predictions.

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A Stability of the steady states

A.1 Trivial steady state

To determine the stability of ϕ_{00}, consider the Jacobian

$$J(\phi_{00}) = \begin{pmatrix} -D & 0 & -r_{11}^s & -r_{21}^s \\ 0 & -D & -r_{12}^s & -r_{22}^s \\ 0 & 0 & r_1^g - D & 0 \\ 0 & 0 & 0 & r_2^g - D \end{pmatrix}.$$

It follows immediately that ϕ_{00} is stable if and only if

$$D > r_1^g|_{\phi_{00}}\,,\ r_2^g|_{\phi_{00}}\,.$$

Since $r_1^g|_{\phi_{00}} = r_1^g(\mathbf{s^f})$ and $r_2^g|_{\phi_{00}} = r_2^g(\mathbf{s^f})$, the stability condition says that the trivial steady state is asymptotically stable if and only if the dilution rate exceeds the specific growth rates of the two species at the feed concentrations.

A.2 Semitrivial steady state

The Jacobian at ϕ_{10} is

$$J(\phi_{10}) = \begin{pmatrix} -D - c_1\frac{\partial r_{11}^s}{\partial s_1} & -c_1\frac{\partial r_{11}^s}{\partial s_2} & -r_{11}^s & -r_{21}^s \\ -c_1\frac{\partial r_{12}^s}{\partial s_1} & -D - c_1\frac{\partial r_{12}^s}{\partial s_2} & -r_{12}^s & -r_{22}^s \\ c_1\frac{\partial r_1^g}{\partial s_1} & c_1\frac{\partial r_1^g}{\partial s_2} & 0 & 0 \\ 0 & 0 & 0 & r_2^g - D \end{pmatrix}.$$

One of the eigenvalues of $J(\phi_{10})$ is

$$\lambda_2 = r_2^g|_{\phi_{01}} - D. \tag{19}$$

The sign of this eigenvalue tells us whether species 2 is capable of invading the chemostat when it is operating at the steady state, ϕ_{10}. In particular, $\lambda_1 < 0$ if and only if $r_2^g|_{\phi_{01}} < D$, i.e., species 2 cannot invade the chemostat. However, this condition, by itself, cannot ensure that ϕ_{10} is observable, since the steady state might be unstable even if we deliberately exclude the possibility of an invasion by species 2 by performing a single-species experiment with species 1 at the same D and $\mathbf{s_f}$. The remaining three eigenvalues of $J(\phi_{10})$ tell us if ϕ_{10} is stable in such single-species experiments. Consistent with this argument, the remaining three eigenvalues of $J(\phi_{10})$ are, in fact, the eigenvalues of its submatrix

$$A = \begin{pmatrix} -D - c_1\frac{\partial r_{11}^s}{\partial s_1} & -c_1\frac{\partial r_{11}^s}{\partial s_2} & -r_{11}^s \\ -c_1\frac{\partial r_{12}^s}{\partial s_1} & -D - c_1\frac{\partial r_{12}^s}{\partial s_2} & -r_{12}^s \\ c_1\frac{\partial r_1^g}{\partial s_1} & c_1\frac{\partial r_1^g}{\partial s_2} & 0 \end{pmatrix}$$

obtained by ignoring the existence of the second species.

One of the eigenvalues of A is $-D$, i.e.,

$$\lambda_2 = -D. \tag{20}$$

This is a consequence of the following "stoichiometric" relationship resulting from the assumption of constant yields

$$\frac{d}{dt}\left(Y_{11}s_1 + Y_{12}s_2 + c\right) = D\left(Y_{11}s_1^f + Y_{12}s_2^f\right) - D\left(Y_{11}s_1 + Y_{12}s_2 + c\right).$$

The signs of the remaining two eigenvalues of A, namely, can be determined from the growth isocline and consumption curve for species 1. To see this, observe that

$$\mathrm{tr}A = \lambda_2 + \lambda_3 + \lambda_4 = -2D - c_1\frac{\partial r_{11}^s}{\partial s_1} - c_1\frac{\partial r_{12}^s}{\partial s_2}.$$

It can also be shown that

$$\det A = \lambda_2\lambda_3\lambda_4 = -c_1 D \nabla r_1^g \cdot \mathbf{t}_1$$

where $\mathbf{t}_1$ denotes the tangent at ϕ_{10} to the consumption curve, $\Phi_1(\mathbf{s_f})$, oriented along the direction of increasing s_j. Since $\lambda_2 = D$, we obtain

$$\lambda_3 + \lambda_4 = -D - c_1 \frac{\partial r_{11}^s}{\partial s_1} - c_1 \frac{\partial r_{12}^s}{\partial s_2} < 0, \tag{21}$$

$$\lambda_3\lambda_4 = c_1 \nabla r_1^g \cdot \mathbf{t}_1 \tag{22}$$

so that the real parts of λ_3, λ_4 are negative if and only if $\nabla r_1^g \cdot \mathbf{t}_1 > 0$, i.e., the angle between ∇r_1^g and $\mathbf{t}_1$ is less than $\pi/2$. Taken together, the relations, (19–22) imply that ϕ_{10} is stable if and only if $r_2^g|_{\phi_{10}} < D$ and the angle between ∇r_1^g and $\mathbf{t}_1$ is less than $\pi/2$.

A similar argument shows that ϕ_{01} is stable if and only if $r_1^g|_{\phi_{01}} < D$ and $\nabla r_2^g \cdot \mathbf{t}_2 > 0$, where $\mathbf{t}_2$ is the tangent to the consumption curve at ϕ_{01}.

A.3 Nontrivial steady state

The Jacobian at ϕ_{11} is

$$J(\phi_{11}) = \begin{pmatrix} -D - c_1 \frac{\partial r_{11}^s}{\partial s_1} - c_2 \frac{\partial r_{21}^s}{\partial s_1} & -c_1 \frac{\partial r_{11}^s}{\partial s_2} - c_2 \frac{\partial r_{21}^s}{\partial s_2} & -r_{11}^s & -r_{21}^s \\ -c_1 \frac{\partial r_{12}^s}{\partial s_1} - c_2 \frac{\partial r_{21}^s}{\partial s_2} & -D - c_1 \frac{\partial r_{11}^s}{\partial s_1} - c_2 \frac{\partial r_{21}^s}{\partial s_2} & -r_{12}^s & -r_{22}^s \\ c_1 \frac{\partial r_1^g}{\partial s_1} & c_1 \frac{\partial r_1^g}{\partial s_2} & 0 & 0 \\ c_2 \frac{\partial r_2^g}{\partial s_1} & c_2 \frac{\partial r_2^g}{\partial s_2} & 0 & 0 \end{pmatrix}.$$

It follows

$$\det\ J(\phi_{11}) = c_1 c_2 \det(\mathbf{r_1^s}, \mathbf{r_2^s}) \cdot \det(\nabla r_1^g, \nabla r_2^g).$$

Since $c_1, c_2 > 0$ at ϕ_{11}, we conclude that a nontrivial steady state is stable only if

$$\det(\mathbf{r_1^s}, \mathbf{r_2^s}) \cdot \det(\nabla r_1^g, \nabla r_2^g) > 0 \tag{23}$$

i.e., $\det(\mathbf{r_1^s}, \mathbf{r_2^s})$ and $\det(\nabla r_1^g, \nabla r_2^g)$ have the same sign, i.e., $(\mathbf{r_1^s}, \mathbf{r_2^s})$ and $(\nabla r_1^g, \nabla r_2^g)$, have the same orientations.

Note that the foregoing stability criterion is only a *necessary* condition for stability of a coexistence steady state [20]. Hence, coexistence steady states at which $(\mathbf{r_1^s}, \mathbf{r_2^s})$ and $(\nabla r_1^g, \nabla r_2^g)$, have opposite orientations are certainly unstable. However, coexistence steady states at which $(\mathbf{r_1^s}, \mathbf{r_2^s})$ and $(\nabla r_1^g, \nabla r_2^g)$, have the same orientation may or may not be stable.

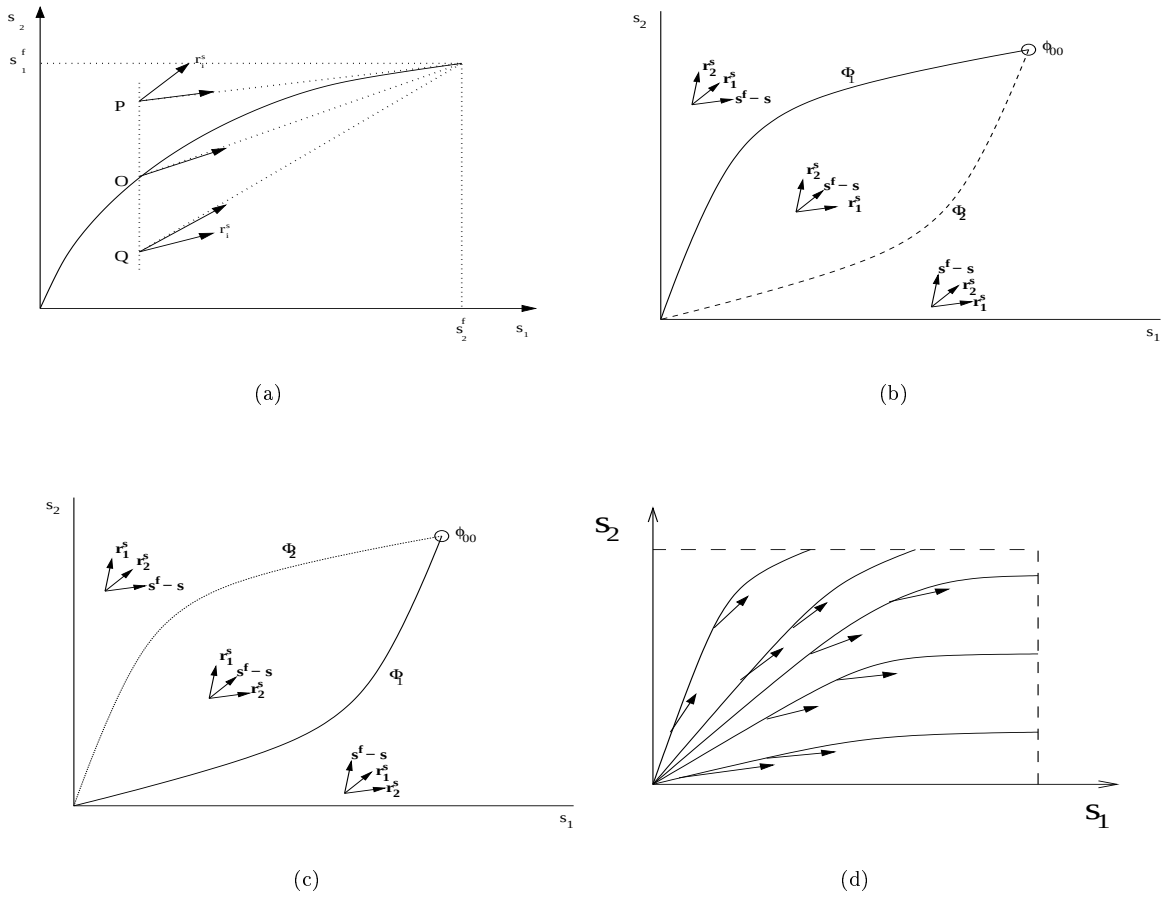
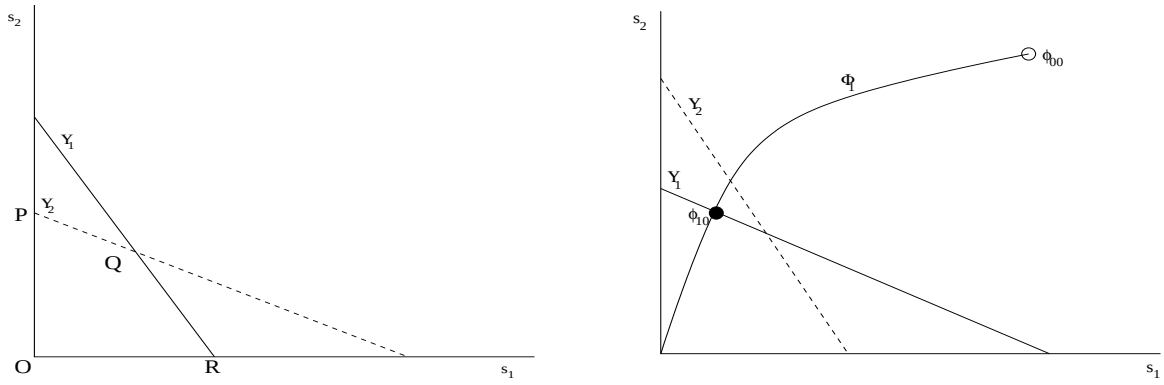


Figure 6: Geometry of the consumption curves: (a) At each point of a consumption curve, the specific substrate uptake vector, $\mathbf{r_i^s(s)}$, is parallel to the substrate depletion vector, $\triangle(\mathbf{s})$. At substrate concentrations above the consumption curve, $\mathbf{r_i^s(s)}$ is above $\triangle(\mathbf{s})$; at substrate concentrations below the consumption curve, $\mathbf{r_i^s(s)}$ is below $\triangle(\mathbf{s})$. (b, c) The substrate depletion vector, $\triangle(\mathbf{s}) \equiv \mathbf{s^f} - \mathbf{s}$, lies between the consumption vectors, $\mathbf{r_1^s(s)}$ and $\mathbf{r_2^s(s)}$ at precisely those substrate concentrations that lie in the region enclosed by the consumption curves for the two species denoted Φ_1 and Φ_2, respectively. The two figures depict the orientations of the vectors when Φ_1 lies above Φ_2 and Φ_2 lies above Φ_1. (d) A family of consumption curves (full lines) and associated specific substrate consumption vectors constructed by performing single-species experiments at various feed concentrations (dashed lines).



(a) Trivial steady state

(b) Semitrivial steady state

Figure 7: Existence and stability of the trivial and semitrivial steady states: (a) The trivial steady state exists for all D and $\mathbf{s^f}$. It is stable precisely when the feed concentrations are in the region OPQR lying below the growth isoclines for both species. (b) The semitrivial steady state, ϕ_{10}, lies at the intersection of the growth isocline, $\Upsilon_1(D)$, and the consumption curve, $\Phi_1(\mathbf{s^f})$, for species 1. It is stable precisely when it lies below $\Upsilon_2(D)$, the growth isocline for species two.

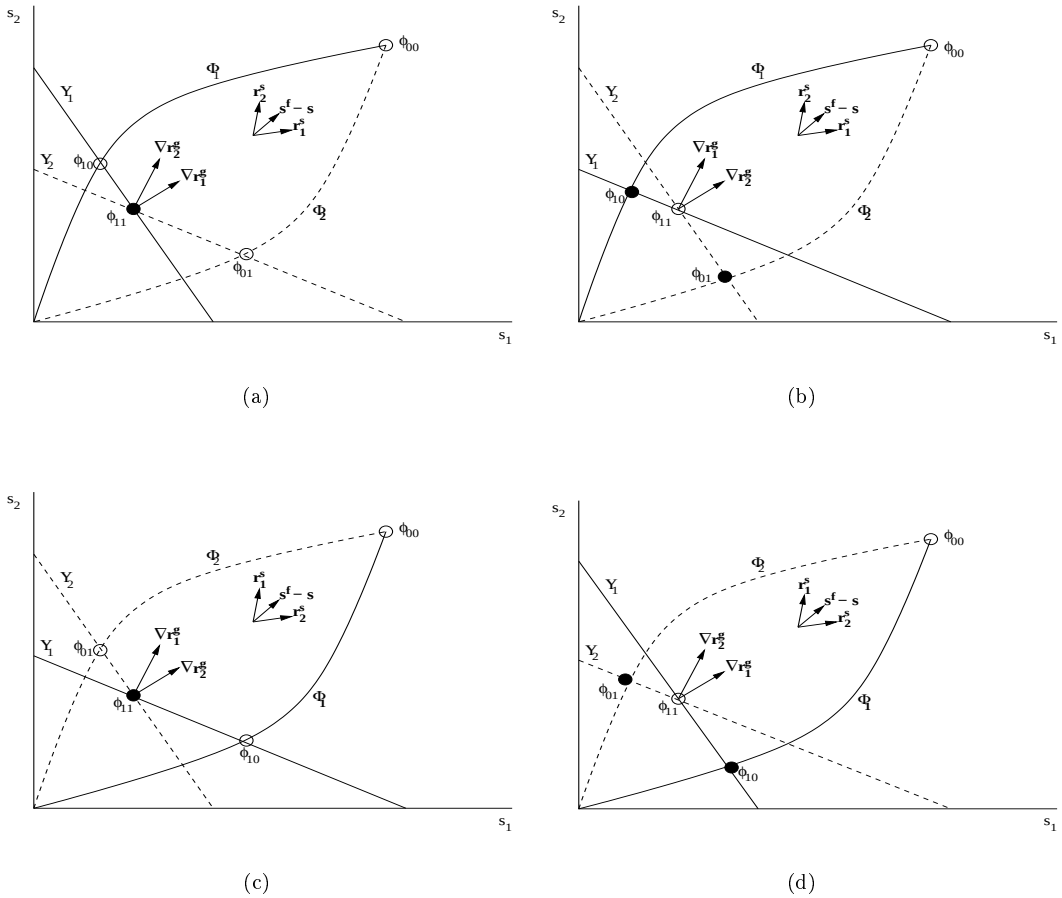


Figure 8: Inferring the stability of the mixed-culture steady states at fixed D and $\mathbf{s^f}$: The growth isocline and consumption curve for species 1, denoted Φ_1 and Υ_1 are shown as full lines. The growth isocline and consumption curve for species 2, denoted Φ_2 and Υ_2 are shown as dashed lines. Stable and unstable steady states are shown as full and open circles, respectively.

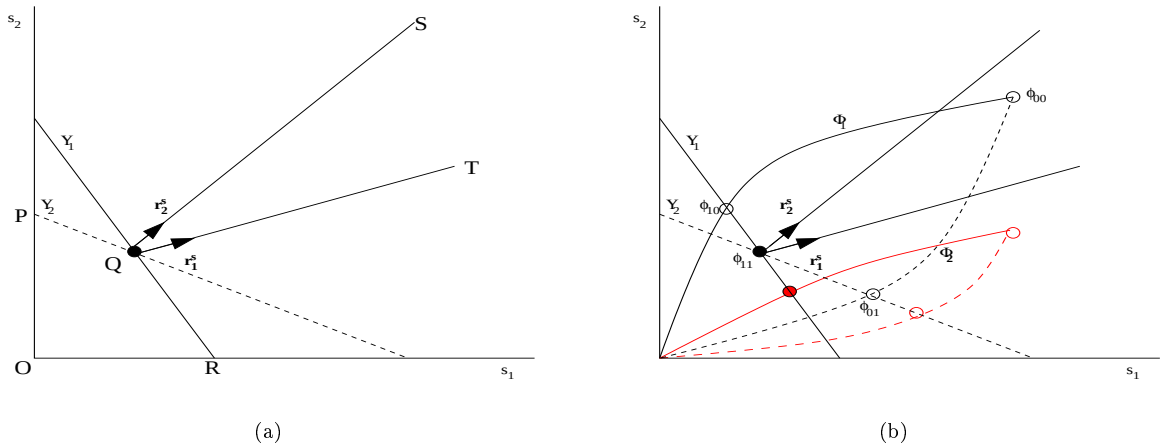


Figure 9: Inferring the stability of the mixed-culture steady states at fixed D and varying $\mathbf{s^f}$: (a) The properties of the steady states at various feed concentrations can be inferred by drawing the cone, SQT, generated by the specific substrate consumption vectors, $\mathbf{r_1^s}$ and $\mathbf{r_2^s}$, at the coexistence steady state. Coexistence is stable if the feed concentrations lie in the cone, SQT. Both species wash out if the feed concentrations lie in the region, OPQR. Only one of the species survives if the feed concentrations lie in the region, PQS, or RQT. (b) Explanation of the results in (a) based on the consumption curves. If s_2^f is decreased while D and s_1^f are fixed, the consumption curves for both species move down. Consequently, the coexistence steady state, ϕ_{11}, is pushed out of the envelope of coexistence. At these new feed concentrations, only species 1 survive will survive in the chemostat.